# Gel/PCR DNA Fragments Extraction Kit

*For research use only*

Geneaid

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

**Sample:** up to 300 mg of agarose gel, up to 100 µl of PCR products

**Fragment Size:** 70 bp – 20 kb

**Recovery:** up to 95%

**Format:** spin column

**Operation Time:** 20 minutes (gel extraction), 10 minutes (PCR cleanup)

**Elution Volume:** 20-50 µl

**Storage:** dry at room temperature (15-25ºC) for at least 1 year without showing any reduction in performance

## Introduction

The Gel/PCR DNA Fragments Extraction Kit was designed to recover or concentrate DNA fragments (70 bp-20 kb) from agarose gel, PCR, or other enzymatic reactions in one convenient product. Chaotropic salt is used to dissolve agarose gel and denature enzymes. DNA fragments in chaotropic salt are bound by the glass fiber matrix of the spin column (1). Contaminants are removed with a Wash Buffer (containing ethanol) and the purified DNA fragments are eluted by a low salt Elution Buffer or TE. Salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture, without phenol extraction or alcohol precipitation. Typically, recoveries are up to 90% for Gel Extraction and up to 95% for PCR Clean Up. The eluted DNA is ready for use in PCR, Fluorescent or Radioactive Sequencing, Restriction Enzyme Digestion, DNA Labeling and Ligation.

## Quality Control

The quality of the Gel/PCR DNA Fragments Extraction Kit is tested on a lot-to-lot basis by isolating DNA fragments of various sizes from either aqueous solutions or agarose gel. The purified DNA is analyzed by electrophoresis.

## Kit Contents

| Component | DF004 | DF100 | DF300 |
|---|---|---|---|
| DF Buffer | 3 ml | 80 ml | 240 ml |
| W1 Buffer | 2 ml | 45 ml | 130 ml |
| Wash Buffer[1] (Add Ethanol) | 1 ml (4 ml) | 25 ml (100 ml) | 50 ml + 25 ml (200 ml) (100 ml) |
| Elution Buffer | 1 ml | 6 ml | 30 ml |
| DF Columns | 4 | 100 | 300 |
| 2 ml Collection Tubes | 4 | 100 | 300 |

[1]Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use.

## Order Information

| Post Reaction DNA Purification | | |
|---|---|---|
| Product | Package Size | Cat. Number |
| GenepHlow™ Gel Extraction Kit | 100/300 preps | DFG100/300 |
| GenepHlow ™ PCR Cleanup Kit | 100/300 preps | DFC100/300 |
| GenepHlow ™ Gel/PCR Kit | 100/300 preps | DFH100/300 |
| GenepHlow ™ DNA Cleanup Maxi Kit | 10/25 preps | DFM010/025 |
| Small DNA Fragments Extraction Kit | 100/300 preps | DF101/301 |
| Presto™ Max Gel/PCR Kit (Large DNA Fragments) | 100/300 preps | DFL100/300 |
| Presto™ 96 Well PCR Cleanup Kit | 4/10 x 96 preps | 96DFH04/10 |
| Presto™ PCR Cleanup Kit 96 Well Binding Plate | 10 plates | 96DBP01 |
| 96-Well G-50 Gel Filtration Plate | 4/10 x 96 rxns | CGP04/10 |
| Gel Extraction Tool | 25 pcs | GXT025 |

## Caution

DF Buffer contains guanidine thiocyanate. During operation, always wear a lab coat, disposable gloves, and protective goggles.

## References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615

## Gel/PCR DNA Fragments Extraction Kit Functional Test Data

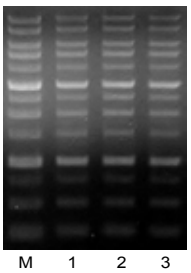



**Figure 1.** Gel slice DNA fragments ranging from 250 bp-10 kb were extracted using the Gel/PCR DNA Fragments Extraction Kit (total 1.0 µg, 3 replicates). The purified DNA from a 50 µl eluate was analyzed by electrophoresis on a 0.8% agarose gel.
M (Control) = Geneaid 1 Kb DNA Ladder

| Test | DNA Conc. | 260/280 | Total DNA | Recovery |
|---|---|---|---|---|
| Control | --- | --- | 1000 ng | --- |
| Test-1 | 17.5 ng/µl | 1.83 | 875 ng | 87.5% |
| Test-2 | 16.4 ng/µl | 1.84 | 820 ng | 82% |
| Test-3 | 17.2 ng/µl | 1.83 | 860 ng | 86% |


**Figure 2.** PCR product DNA fragments ranging from 250 bp-10 kb were extracted using the Gel/PCR DNA Fragments Extraction Kit (total 1.1 µg, 3 replicates). The purified DNA from a 50 µl eluate was confirmed by spectrophotometer and analyzed by electrophoresis on a 1% agarose gel.
M (Control) = Geneaid 1 Kb DNA Ladder

| Test | DNA Conc. | 260/280 | Total DNA | Recovery |
|---|---|---|---|---|
| Control | --- | --- | 1100 ng | --- |
| Test-1 | 20.8 ng/µl | 1.85 | 1040 ng | 95% |
| Test-2 | 20.3 ng/µl | 1.84 | 1015 ng | 92% |
| Test-3 | 19.6 ng/µl | 1.84 | 980 ng | 89% |

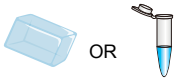
# Gel Extraction Protocol

## IMPORTANT BEFORE USE!

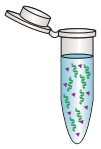
• Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use
• Additional Requirements: absolute ethanol, microcentrifuge tubes

| | |
|---|---|
| Gel Dissociation | • Excise the agarose gel slice containing relevant DNA fragments.<br>• Remove any extra agarose to minimize the size of the gel slice<br>NOTE: Using TAE buffer for gel formation is recommended for optimal DNA recovery.<br>• Transfer **up to 300 mg of the gel slice** to a 1.5 ml microcentrifuge tube.<br>• Add **500 µl of DF Buffer** to the sample and mix by vortex.<br>• Incubate at 55-60ºC for 10-15 minutes to ensure the gel slice has been completely dissolved.<br>NOTE: During incubation, invert the tube every 2-3 minutes.<br>• Cool the dissolved sample mixture to room temperature. |
| Step 1 DNA Binding | • Place the **DF Column** in a **2 ml Collection Tube**.<br>• Transfer **800 µl of the sample mixture** to the **DF Column**.<br>• Centrifuge at 14-16,000 x g for 30 seconds.<br>• Discard the flow-through and place the **DF Column** back in the **2 ml Collection Tube**.<br>NOTE: If the sample mixture is more than 800 µl, repeat the DNA Binding step. |
| Step 2 Wash | • Add **400 µl of W1 Buffer** into the **DF Column**.<br>• Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.<br>• Place the **DF Column** back in the **2 ml Collection Tube**.<br>• Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **DF Column**.<br>• Let stand for 1 minute at room temperature.<br>• Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.<br>• Place the **DF Column** back in the **2 ml Collection Tube**.<br>• Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix. |
| Step 3 DNA Elution | • Transfer the dried **DF Column** to a new 1.5 ml microcentrifuge tube.<br>• Add **20-50 µl of Elution Buffer** or TE into the CENTER of the column matrix.<br>• Let stand for at least 2 minutes to ensure the **Elution Buffer** is completely absorbed.<br>• Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.<br>NOTE: Using pre-heated Elution Buffer (60ºC) is recommended for eluting DNA fragments >8kb. |

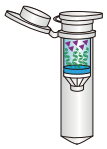
Quick Protocol




Gel Slice PCR Product

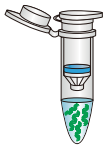


Lysis



DNA Binding

Wash



Elution

# Gel Extraction For Sequencing Protocol

## IMPORTANT BEFORE USE!

• Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use
• Additional Requirements: absolute ethanol, microcentrifuge tubes
• We recommend using the Gel Extraction For Sequencing Protocol as Guanidinium Chloride, a component of W1 Buffer, may interfere with sequencing reactions

| | |
|---|---|
| Gel Dissociation | • Excise the agarose gel slice containing relevant DNA fragments.<br>• Remove any extra agarose to minimize the size of the gel slice<br>NOTE: Using TAE buffer for gel formation is recommended for optimal DNA recovery.<br>• Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube.<br>• Add **500 µl of DF Buffer** to the sample and mix by vortex.<br>• Incubate at 55-60ºC for 10-15 minutes to ensure the gel slice has been completely dissolved.<br>NOTE: During incubation, invert the tube every 2-3 minutes.<br>• Cool the dissolved sample mixture to room temperature. |
| Step 1 DNA Binding | • Place the **DF Column** in a **2 ml Collection Tube**.<br>• Transfer **800 µl of the sample mixture** to the **DF Column**.<br>• Centrifuge at 14-16,000 x g for 30 seconds.<br>• Discard the flow-through and place the **DF Column** back in the **2 ml Collection Tube**.<br>NOTE: If the sample mixture is more than 800 µl, repeat the DNA Binding Step. |
| Step 2 Wash | • Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **DF Column** and let stand for 1 minute.<br>• Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.<br>• Place the **DF Column** back in the **2 ml Collection Tube**.<br>• Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **DF Column** and let stand for 1 minute.<br>• Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.<br>• Place the **DF Column** back in the **2 ml Collection Tube**.<br>• Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix. |
| Step 3 DNA Elution | • Transfer the dried **DF Column** to a new 1.5 ml microcentrifuge tube.<br>• Add **20-50 µl of Elution Buffer** or TE into the CENTER of the column matrix.<br>• Let stand for at least 2 minutes to ensure the **Elution Buffer** is completely absorbed.<br>• Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.<br>NOTE: Using pre-heated Elution Buffer (60ºC) is recommended for eluting DNA fragments >8kb. |

# PCR Clean Up Protocol

## IMPORTANT BEFORE USE!

• Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use
• Additional Requirements: absolute ethanol, microcentrifuge tubes

| | |
|---|---|
| Sample Prep. | • Transfer **up to 100 µl of reaction product** to a 1.5 microcentrifuge tube.<br>• Add **5 volumes of DF Buffer** to 1 volume of the sample and mix by vortex. |
| Step 1<br>DNA Binding | • Place a **DF Column** in a **2 ml Collection Tube**.<br>• Transfer **the sample mixture** to the **DF Column**.<br>• Centrifuge at 14-16,000 x g for 30 seconds.<br>• Discard the flow-through then place the **DF Column** back in the **2 ml Collection Tube**. |
| Step 2<br>Wash | • Add **600 µl of Wash** Buffer **(make sure ethanol was added)** into the CENTER of the **DF Column**.<br>• Let stand for 1 minute at room temperature.<br>• Centrifuge at 14-16,000 x g for 30 seconds.<br>• Discard the flow-through and place the **DF Column** back in the **2 ml Collection Tube**.<br>• Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix. |
| Step 3<br>DNA Elution | • Transfer the dried **DF Column** to a new 1.5 ml microcentrifuge tube.<br>• Add **20-50 µl of Elution Buffer** or TE into the CENTER of the column matrix.<br>• Let stand for at least 2 minutes to ensure the **Elution Buffer** is completely absorbed.<br>• Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.<br>NOTE: Using pre-heated Elution Buffer (60ºC) is recommended for eluting DNA fragments >8kb. |

## Troubleshooting

| Problem | Possible Reasons/Solution |
|---|---|
| Low Yield | **Gel slice did not dissolve completely**<br>• The Gel slice was too big. If using more than 300 mg of gel slice, separate it into multiple tubes.<br>• Raise the incubation temperature to 60ºC and extend the incubation time. |
| | **Incorrect DNA Elution Step**<br>• Ensure that the Elution Buffer is completely absorbed after being added to the center of the DF Column. |
| | **Incomplete DNA Elution**<br>• If the DNA fragments are larger than 8 kb, use pre-heated Elution Buffer (60-70ºC) in the Elution Step to improve the elution efficiency. |
| Eluted DNA doesn't perform well in downstream applications | **Residual ethanol contamination**<br>• Following the Wash Step, dry the DF Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60ºC for 5 minutes. |
| | **DNA was denatured (a smaller band appeared on gel analysis)**<br>• Incubate the eluted DNA at 95ºC for 2 minutes, and then cool down slowly to re-anneal the denatured DNA. |
| Low A260/A230 | • In the wash step, repeat the 600 µl of Wash Buffer addition and let stand for 1 minute. |

# Related DNA Extraction Products

| Plasmid DNA Purification | | |
|---|---|---|
| Product | Package Size | Catalogue Number |
| Presto™ Mini Plasmid Kit | 100/300 preps | PDH100/300 |
| Presto™ Midi Plasmid Kit | 25 preps | PIF025 |
| Presto™ Midi Plasmid Kit (Endotoxin Free) | 25 preps | PIFE25 |
| High-Speed Plasmid Mini Kit (10-50 Kb) | 100/300 preps | PDL100/300 |
| High-Speed Plasmid Advance Kit (50-100 ml) | 25 preps | PA025 |
| Geneaid™ Plasmid Mini Kit | 40/100 preps | PAE040/100 |
| Geneaid™ Plasmid Midi Kit | 25 preps | PI025 |
| Geneaid™ Plasmid Midi Kit (Endotoxin Free) | 25 preps | PIE25 |
| Presto™ Plasmid DNA Concentration Kit | 250/500/1000 preps | PC0250/500/1000 |
| Geneaid™ Plasmid Maxi Kit | 10/25 preps | PM010/25 |
| Geneaid™ Plasmid Maxi Kit (Endotoxin Free) | 10/25 preps | PME10/25 |
| Presto™ 96 Well Plasmid Kit | 4/10 x 96 preps | 96PDV04/10, 96PDC04/10 |
| Presto™ Plasmid 96 Well Binding Plate | 10 plates | 96PBP01 |
| Presto™ Plasmid 96 Well FIlter Plate | 10 plates | 96PFP01 |
| **Post Reaction DNA Purification** | | |
| Product | Package Size | Catalogue Number |
| GenepHlow™ Gel Extraction Kit | 100/300 preps | DFG100/300 |
| GenepHlow ™ PCR Cleanup Kit | 100/300 preps | DFC100/300 |
| GenepHlow ™ Gel/PCR Kit | 100/300 preps | DFH100/300 |
| GenepHlow ™ DNA Cleanup Maxi Kit | 10/25 preps | DFM010/025 |
| Small DNA Fragments Extraction Kit | 100/300 preps | DF101/301 |
| Presto™ Max Gel/PCR Kit (Large DNA Fragments) | 100/300 preps | DFL100/300 |
| Presto™ 96 Well PCR Cleanup Kit | 4/10 x 96 preps | 96DFH04/10 |
| Presto™ PCR Cleanup Kit 96 Well Binding Plate | 10 plates | 96DBP01 |
| DNA Pure Kit | 100/300 preps | DP100/300 |
| G-25 Gel Filtration Desalting Column | 50 rxns | CG025 |
| G-50 Gel Filtration Dye Terminator Removal Column | 50 rxns | CG050 |
| 96-Well G-50 Gel Filtration Plate | 4/10 x 96 rxns | CGP04/10 |
| Gel Extraction Tool | 25 pcs | GXT025 |
| **Genomic DNA Extraction and Purification** | | |
| Product | Package Size | Catalogue Number |
| Genomic DNA Mini Kit (Blood/Cultured Cell) | 100/300 preps | GB100/300 |
| Genomic DNA Midi Kit (Blood/Cultured Cell) | 25 preps | GDI25 |
| Genomic DNA Maxi Kit (Blood/Cultured Cell) | 10/25 preps | GDM10/25 |
| Genomic DNA Mini Kit (Tissue) | 50/100/300 preps | GT050/100/300 |
| gSYNC™ DNA Extraction Kit | 100/300 preps | GS100/300 |
| Genomic DNA Mini Kit (Plant) | 100 preps | GP100 |
| Genomic DNA Maxi Kit (Plant) | 10/25 preps | GPM10/25 |
| Geneaid™ DNA Isolation Kit (Blood) | 100/1,000 rxns | GEB100/01K(+) |
| Geneaid™ DNA Isolation Kit (Bacteria) | 300/3,000 rxns | GEE300/03K(+) |
| Geneaid™ DNA Isolation Kit (Tissue) | 150/1,500 rxns | GET150/1.5K(+) |
| Geneaid™ DNA Isolation Kit (Cultured Cell) | 150/1,500 rxns | GEC150/1.5K(+) |
| GENEzol™ DNA Reagent Plant | 100/200 rxns | GR100/200 |
| Presto™ Mini gDNA Yeast Kit | 100/300 preps | GBY100/300 |
| Presto™ Mini gDNA Bacteria Kit | 100/300 preps | GBB100/101/300/301 |
| Geneius™ Micro DNA Extraction Kit | 100/300 preps | GMB100/300 |
| Presto™ Buccal Swab gDNA Extraction Kit | 100/300 preps | GSK100/300 |
| **DNA RNA Purification** | | |
| Product | Package Size | Catalogue Number |
| Presto™ DNA RNA Extraction Kit | 50/100 preps | DR050/100 |

For additional product information please visit www.geneaid.com. Thank you!